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ORIGINAL PAPER

Calophyllum inophyllum: recalcitrant or intermediate seed?

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Abstract: We studied seed storage behaviour of a multipurpose tree *Calophyllum inophyllum* (Clusiaseae). Seeds were collected at Roslyn bay (23°7′60″ S, 150°43'60″ E) Central Queensland. Seed drying and desiccation tolerance tests were carried out according to International Seed Testing Association (ISTA) rules. We found *C. inophyllum* seeds to be recalcitrant and vulnerable to chilling injury, hence, unsuited for cold storage. *C. inophyllum* seeds maintained their viability for an appreciable period (> 8 months) if stored in warmer environments.

Keywords: *Calophyllum inophyllum*; germination; moisture content; recalcitrant; storage longevity; seed storage behaviour

Introduction

Calophyllum inophyllum L. (Clusiaceae), commonly known as Alexandrian laurel, beauty leaf or Domba (in Sri Lanka), is a native Australian coastal tree species of wide distribution from northern Australia and extending throughout Southeast Asia and southern India (Agroforestry Tree Database 2006). It is a medium to large evergreen tree that grows up to 20 m in height with a broad spreading crown of irregular branches. The tree bears a dense canopy of glossy, elliptical leaves, fragrant white flowers, and large round nuts (Friday & Okano 2006). The species has many economic uses. *C. inophyllum* kernels contain 60%–65% inedible oil and have been identified as one of the potential biodiesel feedstock plants (Azam et al. 2005; Sahoo et al. 2006; Hathurusingha and Ashwath 2007). Kernel oil has a high commercial demand for pharmaceutical and cosmetic applications (Dweck and Meadows 2002). The timber is durable and used in light construction; it has a competitive market value (Little and Skolmen 1989). Moreover, scientists have recently discovered anti-cancer and anti-HIV compounds from *C. inophyllum* extracts (Patil et al. 1993; Taylor et al. 1994; Spino et al. 1998; Itoigawa et al. 2001; Powar et al. 2007).

Seed storage behaviour is an important factor in deciding the economic potential of a crop. It provides a vital prescription for storing seed material for commercial plantation programs. Generally seeds demonstrate three types of storage behaviours: orthodox, intermediate, and recalcitrant (Song et al. 2003; Tweddle et al. 2003). Seeds that can be dried to moisture contents between 6% and 10% and stored successfully at low temperatures have been described as “orthodox” in storage behaviour, while seeds that cannot be dried to moisture levels below 20% without losing viability have been described as “recalcitrant” (Roberts 1973). Seeds with storage characteristics between these extremes are categorised as “intermediate” seeds. While these terms have been considered as less than ideal choices to describe physiological behaviour of seeds (Berjaket et al. 1990), they have been widely accepted and are commonly used by most seed scientists (Bonner 1995).

Published accounts of the seed storage behaviour of *C. inophyllum* are inconclusive. The seed information database (SID 5.0) of the Royal Botanic Gardens, Kew UK also does not provide clear information about the storage behaviour of *C. inophyllum*. Different authors have reached different conclusions; for example, Ng (1992) and Gupta et al. (2009) reported that seed storage behaviour of *C. inophyllum* is intermediate. However, Allen (2002) suggested that *C. inophyllum* seeds are recalcitrant. We undertook this study to determine the storage behaviour of *C. inophyllum* seeds found in Australia by investigating their desiccation tolerance and storage longevity in three different environments.

Materials and methods

Desiccation treatments

We collected mature seeds (with no exocarps) from three eight-year-old trees in roadside plantings at Roslyn bay, Yeppoon (23°7'60″ S, 150°43'60″ E) during October 2007. One hundred and thirty seeds free of exocarp were used for the experiment.

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Ten randomly selected seeds were used to estimate initial moisture content of the seed sample. They were weighed (W1) and then oven dried at 105°C for 17 h (ISTA, 1979) and again weighed (W2). Initial moisture content was estimated by the following equation.

$$MCi = (W1 - W2) / W1 \times 100 \quad (1)$$

where *MCi* is the moisture content, *W*1 the fresh weight of the seed, and *W*2 is the dry weight of the seed. Initial moisture content of the selected lot of 10 seeds was 40%.

The method of the International Seed Testing Association (ISTA 1999) was used to dry seeds to decreasing levels of moisture content. Silica gel was used in drying seeds. Seeds (60 each) were kept in two desiccators (6 L capacity) containing silica gel (equal to 75% of the weight of seeds). Temperature was maintained at 25°C (Hong et al. 1996) and vacuum pumps were connected to desiccators in order to induce rapid drying.

Numbers of desiccation treatments were reduced to suit the sample size. Seeds of declining moisture contents (38%, 22%, 18%, and 14%) were obtained by removing seeds at timed intervals. At each of the above four moisture levels, 15 seeds were separated from the sample and kept in sealed plastic bags to prevent further drying. Plastic bags were weighed at the time of transfer and reweighed before germination tests were carried out to check for any moisture loss.

Germination test I

A germination test was carried out to test the viability of the seed samples at each of the four moisture levels. Twelve pots (20 cm in diameter) were filled with sterilized river sand and three pots (replicates) were used to test germination at each moisture content level (38%, 22%, 18%, and 14%). Ten seeds from each treatment were planted in each pot (30 seeds in total per moisture level). Shells were slightly cracked by using a mechanical vice before planting to shorten the germination period (Parras 1939). Pots were placed in a shaded and auto irrigated rack. Auto sprinkling was set at 15 mins/twice a day. After 30 days, the number of germinated seeds was counted. Counting was stopped after 65 days. All ungerminated seeds were tested for viability using a tetrazolium chloride (TTC) stain. Germination percentage (GP) and mean germination time (MGT) were calculated using the following equations:

$$GP(\%) = (N / S) \times 100\% \quad (2)$$

where *N* is the total number of germinated seeds and *S* is the total number of seeds.

$$MGT(d) = \sum (t_i \times n_i) / N \quad (3)$$

where t_i is the *i*th day from sowing, n_i is the number of germinated seeds at the *i*th day from sowing and *N* is the total number of germinated seeds (Liu et al. 2005).

Storage longevity

Mature seeds devoid of exocarp were collected from three eight-year-old trees from roadside plantings at Roslyn Bay, Yeppoon (23°7′60″ S, 150°43'60″ E) during October 2007. Three hundred and seventy (370) mature seeds were used in this experiment. Initial moisture content was estimated for 10 randomly selected seeds as described above. Fresh seeds were dried up to 20% moisture content by using Silica gel (equal to approximately 75% of the weight of the seeds) in a sealed plastic container at 25°C and immediately transferred to three porous sterilized cardboard boxes (30 cm × 45 cm × 15 cm). Weights of the boxes were recorded (W1, W2, W3). Boxes were then placed in different temperature environments: cool room (T ≈ 8°C, RH ≈ 55–60%), potting shed (T≈25–40°C, RH 50–75%), and eco-physiology lab (T≈25°C, RH ≈50–65%).

Germination test II

After every three months of storing seeds in above conditions, 30 seeds (3 replicates × 10 seeds) were removed from each box for germination tests. Seeds from three storage environments were planted in (2 reps × 3) pots (20 cm in diameter) containing washed river sand. Shells were completely removed (without making any injury to the kernel) before planting to reduce the germination time (Parras 1939). Auto sprinkling was set at 15 mins/twice a day. After 35 days the number of germinated seeds was counted. Germination percentage (GP) and mean germination time (MGT) were calculated using the above equations.

Moisture loss in storage

Four seeds each were placed uncovered in the three selected storage environments and individual weights were recorded for 28 days.

Results and discussion

Desiccation tolerance

The highest germination percentage (80%) was found in seeds that contained 38% moisture content (MC). Fig. 1 shows a significant decline in germination percentage at moisture content below 22%. Germination percentage drastically dropped from 76.7±0.33% to 36.7±0.30% when the moisture content was reduced from 22% to 18%. The results (Fig. 1) indicate that viability of *C. inophyllum* seeds is largely dependent on their moisture content. Scande et al. (2001) also reported that some seeds of tropical origin are susceptible to dehydration. In dry environments, seeds lose their viability due to the shrinkage, aqueous based degradation or denaturation of storage chemicals caused by dehydration (Pammenter and Berjak 1999).

In this experiment less than 50% of the seeds survived at moisture content below 20%. The sharp decline in germination

percentage of *Calophyllum* seeds in response to the reduction of MC from 22%–18% indicates that *C. inophyllum* seeds do not retain viability below a minimum moisture level, which has been described as the CMC "critical moisture content" (King and Roberts 1979) or "lowest safe moisture content" (Tompsett 1984), and is a common characteristic of recalcitrant seeds.

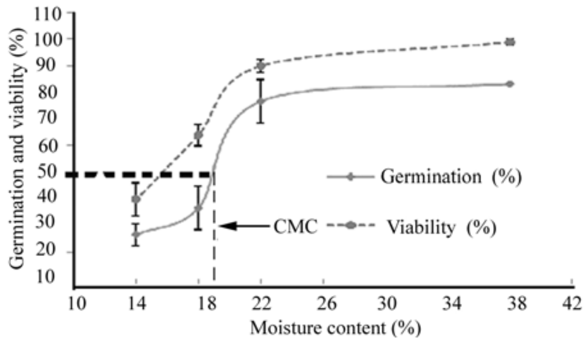

Fig. 1 Seed germination percentage and viability (TTC reduction) of seed samples at different moisture levels. *CMC- critical moisture content, n=3.

Based on a CMC of 18%–22%, our data suggest that *C. inophyllum* seeds are recalcitrant, as reported by Allen (2002). Our results (Fig. 2) also show that reduction in seed moisture content can prolong germination. Mean germination time (MGT) increased with declining seed moisture content to 18%, but did not increase further in drier seed.

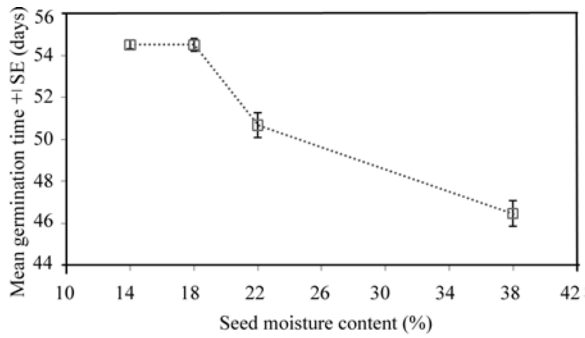

Fig. 2 Mean germination time of *Calophyllum inophyllum* seeds with different moisture contents, n=3.

Storage longevity

During the eight months of storage, the majority of *Calophyllum* seeds retained their viability in the warmer (25–30°C) and relatively humid storage environments (Fig. 3). In contrast to our findings, Foxworthy (1927) reported that *C. inophyllum* seeds generally do not maintain their viability for long periods. Gupta et al. (2009) also reported that *C. inophyllum* seeds have very short storage longevity (less than a fortnight) when kept at ambient temperatures (≈25°C). However, our study showed that in warm (25–30°C) and humid environments, more than 70% of *C. inophyllum* seeds maintained viability for more than eight months. Seeds of related species (*Calophyllum calaba*) reportedly are able to maintain viability up to one year if stored in a dry room (Weaver 1990).

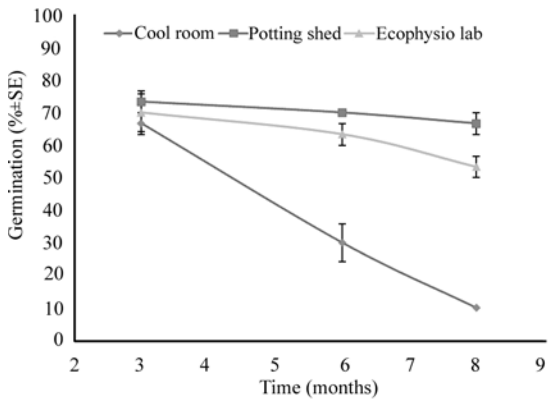

Fig. 3 Germination percentage of *C. inophyllum* seeds stored under different conditions: Potting shed (≈25–40°C, RH ≈50–75%), Ecophysiology lab (≈25°C, RH≈ 50–65%) and Cool room (≈8°C, RH ≈60%). n=3.

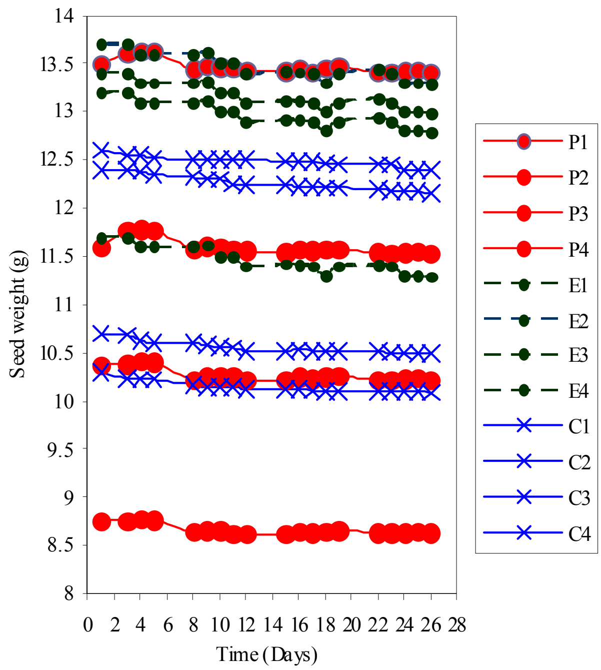

Fig. 4 Changes in the weight of four *Calophyllum inophyllum* seeds placed in different environments over time. P-seeds in the potting shed, E-seeds in the ecophysiology lab, C-seeds in the cool room.

Shorter storage longevity of *Calophyllum* seeds reported by Gupta et al. (2009) might have been due to complete removal of the endocarp, which subjected the seeds to rapid desiccation. In

humid and warmer storage environments, seeds with intact endocarp resisted desiccation to CMC for a slightly longer period (> 8 months).

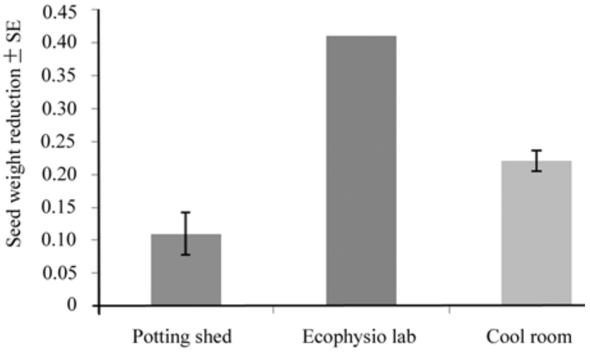

Fig. 5 Seed weight reduction in *Calophyllum* seeds after 26 days in different environments (n=4).

Calophyllum seeds stored in a potting shed appeared to periodically re-hydrate while steady reduction in seed weight was observed among seeds stored in a cool room (Fig. 4). Slowest moisture loss was observed in seeds stored in the potting shed followed by those stored in the cool room (Fig. 5).

Moisture loss in seeds stored in the potting shed (25–40°C, RH ≈59%–75%) was slower than for seeds stored in the ecophysiology lab (≈25°C, RH ≈50%–65%) and the seeds stored at ecophysiology lab recorded lower germination percentage at the selected intervals compared to those stored in the potting shed. This shows that moisture loss in dry environments has some influence on seed storage longevity.

Seeds stored in the cold room recorded the lowest germination percentage despite experiencing slower moisture loss than did seeds stored in the ecophysiology lab. In agreement with the findings of this study, Gupta et al. (2009) also reported that *Calophyllum* seeds wrapped in moist paper towels in plastic boxes at 4−10°C cold storage environments retained viability only for up to four months. This shows that low temperatures may have a negative influence on seed storage longevity. Careful observation of kernels revealed that under cold/dry (≈8°C /RH ≈55%–60%) storage environments the *Calophyllum* seeds became darker coloured at the centre (Fig. 6), suggesting that low viability of cold-stored seeds might have been caused by chilling injury as reported by Hong et al. (1996).

Some seeds from tropical environments are vulnerable to chilling injury (Corbinearu and Come 1988; Tompsett 1994). These tropical seeds are typically injured when they are stored below 10−15°C (Sacande et al. 2001), partly due to dysfunction of some enzymes (Lyons et al. 1979; Yoshida et al. 1986) and partly due to leakage of cytoplasmic solutes (Bergevin et al. 1993; Bertin et al. 1996). This suggests that darkening of the inner centre parts of the kernels of *Calophyllum* seeds stored in cold storage may be due to leakage of cytoplasmic solutes.

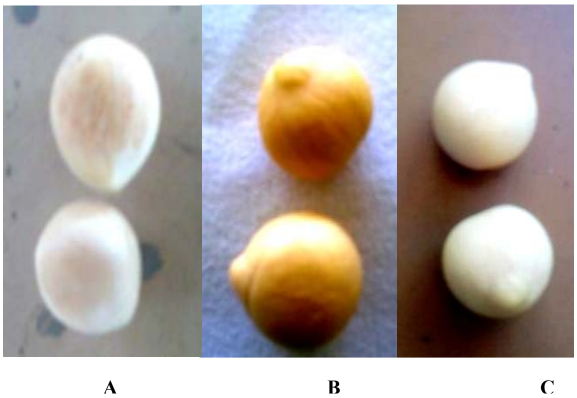

Fig. 6 *Calophyllum* kernels after eight months of storage. A-cold room, B-ecophysiology lab, C-potting shed.

After eight months of storage, seeds stored in the potting shed (25–40°C, RH ≈59%–75%) appeared pale yellow and those stored in the ecophysiogy lab (≈25°C, RH ≈50%–65%) were dark yellow. Seeds stored in the ecophysiology lab had the second highest moisture loss. Dweck and Meadow (2002) noticed darkening of the colour of *Calophyllum* kernels under desiccation. Kernels of *C. inophyllum* contain high amount (111±5 mg/kg) phenolic compounds (Seneviratne and Kotuwegedara 2009). They are known to cause discolouration in vegetables and fruits (Xu and Diosady 2002). Darker colour in kernels that were stored in the ecophysiology lab for eight months might be due to oxidation of these phenolic compounds. In agreement with our findings, Dweck and Meadows (2002) reported that browning of *Calophyllum* seed indicates loss of germinative potential.

Conclusion

C. inophyllum seed appears to have a "critical moisture content" which is a characteristic feature of a recalcitrant seed. *C. inophyllum* seed can retain viability for an appreciable period (> 8 months) if stored in warmer and slightly humid environments without removing the endocarp. *Calophyllum* seeds appear to be sensitive to low temperatures. More focused tests should be carried out to establish the vulnerability of *C. inophyllum* seed to chilling injury.

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